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Current Opinion in Neurobiology

Modeling psychiatric disorders with patient-derived iPSCs

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Psychiatric disorders are heterogeneous disorders characterized by complex genetics, variable symptomatology, and anatomically distributed pathology, all of which present challenges for effective treatment. Current treatments are often blunt tools used to ameliorate the most severe symptoms, often at the risk of disrupting functional neural systems, thus there is a pressing need to develop rational therapeutics. Induced pluripotent stem cells (iPSCs) reprogrammed from patient somatic cells offer an unprecedented opportunity to recapitulate both normal and pathologic human tissue and organ development, and provides new approaches for understanding disease mechanisms and for drug discovery with higher predictability of their effects in humans. Here we review recent progress and challenges in using human iPSCs for modeling neuropsychiatric disorders and developing novel therapeutic strategies.

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Current Opinion in Neurobiology 2016, **36**:118–127

This review comes from a themed issue on **Neurobiology of disease**

Edited by **Dennis J Selkoe** and **Daniel R Weinberger**

For a complete overview see the Issue and the Editorial

Available online 17th December 2015

http://dx.doi.org/10.1016/j.conb.2015.11.003

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Introduction

Severe psychiatric disorders, or mental illnesses, such as schizophrenia (SZ), autistic spectrum disorder (ASD), bipolar disorder (BP), and anxiety disorders, are chronic and complex neurological diseases affecting a large portion of the world's population [1,2]. Currently available drugs are primarily targeted at relieving symptoms, are often only partially effective, and have significant side effects on well-functioning neural systems [3,4]. Thus, development of rational therapeutics based on an understanding of the disease etiology and pathogenesis is imperative.

One major challenge in the investigation of disease mechanisms and drug development for mental disorders is a lack of predictive preclinical models due to the heterogenetic and multifactorial nature of these illnesses. Translating basic findings from animal models into effective therapeutics frequently fails, largely due to developmental, biochemical, metabolic and physiological differences in humans [5]. Patient studies are limited in that postmortem brain tissue represents the disease endpoint and it is difficult to disentangle treatment effects from primary pathology, whereas brain imaging can reveal impairments at the neural system level, but limited information on cellular and molecular pathology. Recently, a paradigm-shift in modeling neurological disorders has emerged based on cellular reprogramming of adult somatic cells into human induced pluripotent stem cells (hiPSCs) [6]. HiPSCs can give rise to all human tissues and provide a renewable source of cells, which are genetically identical to the donor. By differentiating patient-derived hiPSCs into disease-relevant cell types, it is now possible to conduct controlled experiments on living neural tissue to study pathogenesis in human cells with disease-permissive genetic contexts [7] (Figure 1). Here, we review recent progress in hiPSC-based modeling of psychiatric disorders and discuss challenges in this rapidly evolving field.

Selection of patient cohorts for disease modeling

Generating hiPSCs for cellular phenotyping of disease-relevant neurons is still an expensive and time-consuming process and careful selection of patient cohorts is critical to yield the most information from relatively small sample sizes. One approach is to stratify patient groups based on genetic risk, which can take the form of many DNA variations, such as single nucleotide polymorphisms (SNPs), copy number variations (CNVs), and small exonic missense and nonsense mutations [1,8]. While rare variants may confer large relative risks (e.g. CNVs), a combination of common variants (e.g. SNPs) with modest individual effect sizes occurs more frequently and can result in a significant cumulative risk load [9,10]. A key question is to what extent these different kinds of genetic risk factors may lead to convergent phenotypes and affect similar biological processes. With respect to patient cohort

Figure 1

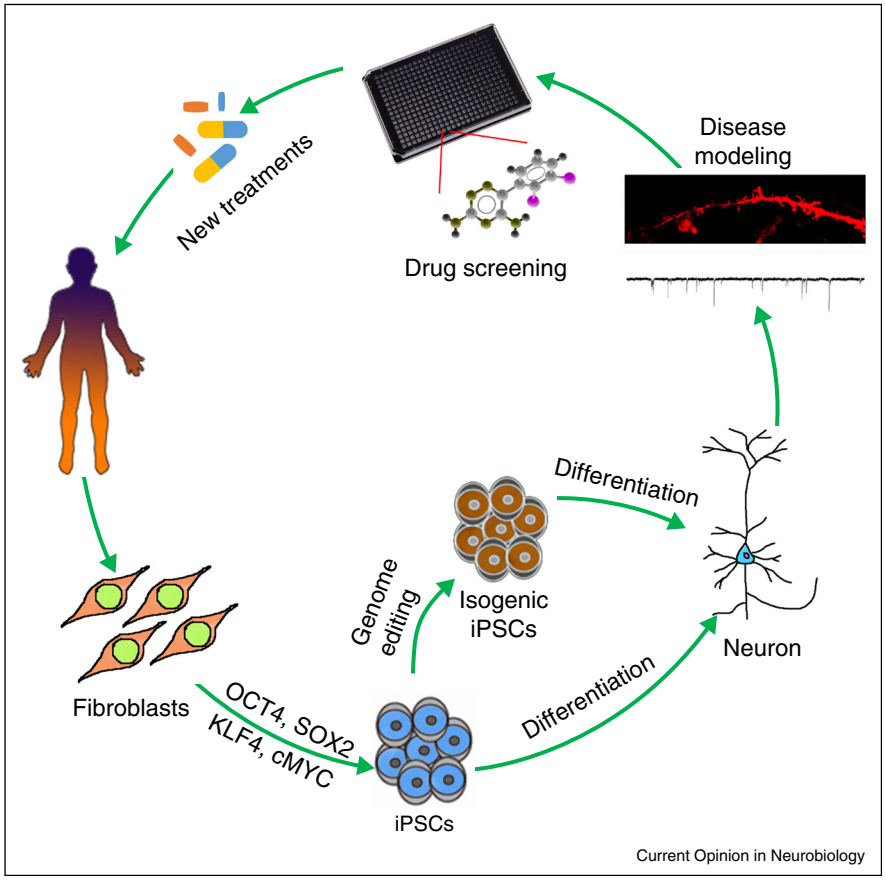

Diagram of disease modeling and drug development with human iPSCs. Patient specific iPSCs could be derived from skin biopsies of patients with psychiatric disorders by ectopic co-expression of four Yamanaka factors. In cases in which the genetic mutation is known, isogenic iPSC lines, either correcting the mutation in the patient iPSCs or introducing the mutation into healthy control iPSCs, could be generated by genome editing techniques, such as CRISPR/Cas9 or TALEN. Human iPSCs could be further differentiated into the affected neuronal subtypes (e.g. cortical glutamatergic neurons) for disease modeling *in vitro*. The identified phenotypes in patient iPSC-derived neurons could be used as readouts for high-throughput drug screening, which would facilitate the discovery of novel therapeutic compounds to treat psychiatric disorders.

selection for hiPSC studies, there are advantages and disadvantages of studying different kinds of genetic risk as outlined below.

Modeling cohorts with rare, highly penetrant mutations

Highly penetrant, well-established mutations offer a clear point-of-entry to begin to investigate the effect of genetic risk on cellular development and function. Rare, multiply affected families in which a single genetic locus likely confers susceptibility for several related disorders have proven invaluable in establishing a link between genetic risk and neuronal dysregulation. For example, Disrupted-in-schizophrenia 1 (DISC1) was initially identified in a large Scottish family harboring an chromosomal translocation that segregates with SZ, BP and major depression [11]. A 4 base-pair (bp) deletion in DISC1 was later discovered to co-segregate with major psychiatric disorders in a smaller American family [12]. By generating iPSC lines from multiple family members of this pedigree, as well as isogeneic lines via genome editing, and differentiating these iPSCs into forebrain cortical neurons, Wen and colleagues found that mutant DISC1 causes aberrant synaptic formation and synaptic vesicle release deficits, as well as transcriptional dysregulation of many genes related to synapses and psychiatric disorders (Table 1) [13••]. This is also a clear illustration of how a specific genetic mutation can be causal for a particular cellular phenotype, but not causal for the disorder in the affected family.

ASD is a complex group of disorders with a strong genetic component, a small subset of which are caused by single gene mutations [2]. HiPSC-derived neurons from patients with Rett syndrome (RTT), a monogenic ASD associated with mutations in methyl CpG binding protein-2 (MECP2), exhibited increased frequency of *de novo* long interspersed nuclear element-1 (L1) retrotransposition, decreased soma size, altered dendritic spine density and reduced excitatory synapses [14–17]; some of

Table 1

Summary of recent studies on iPSC-based modeling of psychiatric disorders

Disease	Genetic variants	Types of cells	Phenotypes	Rescued?	Reference
Schizophrenia	Sporadic	Neurons	Decreased neuronal connectivity; altered gene expressions	Loxapine	Brennand *et al*. [38]
Schizophrenia	Sporadic	Neurons	Altered oxygen metabolism	Valproic acid	Paulsen *et al*. [36]
Schizophrenia	Sporadic	DA/glutamatergic neurons	Abnormal neuronal differentiation and mitochondrial dysfunction	N/A	Robicsek *et al*. [39]
Schizophrenia	DISC1Δ4bp	Cortical glutamatergic neurons	Synaptic deficits in presynaptic vesicle releasing; altered gene expressions	N/A	Wen *et al*. [13••]
Schizophrenia	Sporadic	Hippocampal DG granule neurons	Lowered levels of NEUROD1, PROX1, and TBR1; reduced neuronal activity; reduced levels of spontaneous neurotransmitter release	N/A	Yu *et al*. [41••]
Schizophrenia	22q11 deletions	Neurons	High L1 copy number in SZ neurons; increased L1 copy number after immune activation by poly-I:C or EGF	N/A	Bundo *et al*. [19]
Schizophrenia	15q11.2 deletion	NPCs	Deficits in adherent junctions and apical polarity	N/A	Yoon *et al*. [26••]
Schizophrenia	Sporadic	NPCs	Abnormal responses to environmental stresses	N/A	Hashimoto-Torii *et al*. [40]
Schizophrenia	Sporadic	NPCs	Abnormal gene expression and protein levels related to cytoskeletal remodeling and oxidative stress	N/A	Brennand *et al*. [91]
Bipolar	Sporadic	Neurons	Changes in gene expressions involved in calcium signaling and telencephalic neuronal fate	Lithium	Chen *et al*. [43•]
Bipolar	Sporadic	NPCs	Increased expression of CXCR4; altered gene expression for neural development and plasticity	N/A	Madison *et al*. [42•]
Bipolar	Sporadic	Neurons	Increased levels of miR-34a	N/A	Bavamian *et al*. [92]
Major depression	DISC1Δ4bp	Cortical glutamatergic neurons	Synaptic deficits in presynaptic vesicle releasing; altered gene expressions	N/A	Wen *et al*. [13••,36]
Rett syndrome	MECP2 mutations	Neurons	Decreased soma size; altered dendritic spine density; and reduced excitatory synapses	N/A	Marchetto *et al*. [15]
Rett syndrome	MECP2 mutations	NPCs	Increased frequency of L1 retrotransposition	N/A	Muotri *et al*. [14]
Rett syndrome	MECP2 R294X	Neurons	Decreased soma size	N/A	Ananiev *et al*. [17]
Rett syndrome	MECP2 mutation	Neurons	Decreased soma size	N/A	Cheung *et al*. [16]
Rett-like syndrome	CDKL5 mutations	Neurons	Aberrant dendritic spines	N/A	Ricciardi *et al*. [18]
ASD	TRPC6 mutation	Neurons	Altered neuronal development, morphology and function	IGF-1 or hyperforin	Griesi-Oliveira *et al*. [20]
Timothy syndrome	CACNA1C mutation	Cortical NPCs and neurons	Defects in calcium signaling and activity-dependent gene expression; abnormalities in differentiation	Roscovitine	Pasca *et al*. [22]
Timothy syndrome	CACNA1C mutation	Cortical NPCs and neurons	TS-associated transcriptional changes were co-regulated by calcium-dependent transcriptional regulators	N/A	Tian *et al*. [23]
Phelan-McDermid Syndrome	22q13.3 deletions	Cortical neurons	Deficits in excitatory synaptic transmission	IGF-1	Shcheglovitov *et al*. [29••]
Williams-Beuren syndrome/ 7q-microduplication syndrome	7q11.23 deletions/ duplications	NPCs	Disrupted transcriptional circuits in disease-relevant pathways	N/A	Adamo *et al*. [27•]
ASD/Schizophrenia	Isogenic NRXN1 mutations	Cortical glutamatergic neurons	Impaired neurotransmitter release	N/A	Pak *et al*. [33]

these phenotypes have been also observed in hiPSC-derived neurons of patients with Rett-like syndrome due to mutations in cyclin-dependent kinase-like 5 [18] and SZ patients with 22q11 deletion [19]. Recently, a *de novo* mutation in TRPC6, a cation channel, has been reported in a non-syndromic autistic individual [20]. In hiPSC-derived neurons from this patient, TRPC6 reduction or haploinsufficiency leads to altered neuronal development and function. Interestingly, MeCP2 levels affect TRPC6 expression, revealing potential common biological pathways among ASDs. Timothy syndrome (TS), one of the most penetrant forms of ASD, is caused by a point mutation in the L-type voltage-gated Ca^{2+} channel encoded by the CACNA1C gene [21]. TS hiPSC-derived cortical neural progenitor cells (NPCs) and neurons show aberrant Ca^{2+} signaling, which could be ameliorated by treatment with roscovitine, a cyclin-dependent kinase inhibitor and atypical L-type Ca^{2+} channel blocker [22]. TS-associated transcriptional changes were predicted to be co-regulated by Ca^{2+}-dependent transcriptional regulators, including NFAT, MEF2, CREB, and FOXO, thus providing a mechanism by which altered Ca^{2+} signaling in TS patients leads to transcriptional dysregulation [23].

Modeling cohorts with large CNVs

Several large, rare CNVs are strongly associated with developmental and psychiatric disorders [24], but are among the most difficult to model in animals since they represent large-scale modifications of DNA that can encompass many genes. Although systematic analysis is still required to pinpoint the gene(s) responsible for conferring risk for psychiatric disorders, hiPSC-based studies are a more tractable system in which to investigate the role of individual genes, which can then be further tested in animal models. One such example is the 15q11.2 BP1–BP2 microdeletion, which is associated with increased risk for SZ, ASD and epilepsy [25]. HiPSC-derived NPCs from patients carrying 15q11.2 microdeletions were found to exhibit deficits in adherens junctions and apical polarity [26••]. Haploinsufficiency of cytoplasmic FMR1-interacting protein 1 (CYFIP1), one of the genes within 15q11.2, was determined to be responsible for these defects by altering cytoskeletal dynamics. Returning to animal models, *in utero* suppression of CYFIP1 expression in the developing mouse neocortex revealed similar defects in radial glial cell polarity and cortical lamination defects, demonstrating how hiPSCs and *in vivo* animal models can provide complementary information and used reciprocally to generate and test new hypotheses. In turn, the identification of signaling pathways in hiPSC and animal models led to identification of an epistatic interaction between CYFIP1 and WAVE signaling mediator ACTR2 in risk for schizophrenia. Recently, Testa and colleagues studied 7q11.23 microdeletions, associated with Williams-Beuren syndrome, and 7q-microduplication syndrome, which display a striking combination of shared and symmetrically opposite phenotypes [27•]. High-resolution, comprehensive molecular analysis revealed that 7q11.23 dosage imbalance disrupts transcriptional circuits in disease-relevant pathways beginning as early as the pluripotent state, which is further amplified upon differentiation into disease-relevant lineages. Another prominent CNV is the 22q11 deletion associated with ~1–2% of SZ patients [28]. HiPSC-derived neurons containing 22q11 deletions showed increased L1 retrotranspositions, similar to RTT patient neurons [19], suggesting that hyperactive retrotransposition of L1 in neurons may contribute to susceptibility for SZ and ASDs. In another example, Phelan-McDermid Syndrome (PMDS) is a neurodevelopmental disorder caused by deletions of 22q13.3. PMDS iPSC-derived neurons were found to have significant deficits in excitatory synaptic transmission that could be corrected by IGF-1 treatment [29••]. Together, several of these hiPSC-based studies support the 'disease of synapses' hypothesis for the biological basis of neuropsychiatric disorders and points of convergence for different genetic risk factors and potential therapeutic targets.

Modeling cohorts with SNP risks via genome editing

Highly penetrant genetic risk factors account for less than 10% of SZ and 15–30% of ASD cases, whereas multiple common variants with small individual effect sizes account for ~30% of the variance in risk for SZ [30]. Recent GWAS have identified many of disease-associated loci for SZ and ASD [9,31], but it is difficult to determine the contribution of these common variants due to modest individual effects and potential epistatic interactions based on genetic background. One way to avoid confounds arising from variable genetic backgrounds is to generate isogenic hiPSC-derived neurons that differ only at the target SNP locus. Genome-editing systems, including designer endonuclease technologies such as zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regulatory interspaced short palindromic repeat (CRISPR)/Cas9 endonuclease, can be used to edit the human genome with relatively high efficiency [32]. Südhof and colleagues recently generated two different heterozygous conditional NRXN1 mutations in human embryonic stem cells (hESCs) [33]. NRXN1 encodes neurexin-1, a presynaptic cell adhesion molecule and both heterozygous NRXN1 mutations impaired neurotransmitter release, but had no effect on synapse formation. This group also generated isogenic hESCs conditionally expressing heterozygous and homozygous mutations in a gene linked to infantile early epileptic encephalopathy, STXBP1 [34], which encodes another presynaptic protein, MUNC18-1. Heterozygous STXBP1 mutations decreased spontaneous and evoked neurotransmitter release, again supporting the role of synaptic development deficits in SZ pathology. Using TALENs and CRISPR/Cas9, Young-Pearse and colleagues disrupted DISC1 near the site of the chromosome translocation found in the Scottish pedigree and

found increased WNT signaling in iPSC-derived NPCs [35]. Notably, while these studies provide important insight into loss-of-function phenotypes and basic biology of these risk genes in human NPCs and neurons, patient-specific mutations may have a different net effect on gene expression, as in the case of the 4 bp deletion in DISC1 that was shown to be a gain-of-function mutation [36]. Isogenic lines are a powerful tool to demonstrate the effect of targeted mutations identified in patient populations, such as CACNA1C, or to selectively manipulate specific genes to determine disease-relevant loci within larger risk-associated CNVs [37].

Modeling idiopathic cohorts

For most patients, however, the contributing genetic factors are unknown. Taking an unbiased approach to cellular phenotyping in hiPSC-derived neurons from these patients may reveal common pathways, biological processes, disease mechanisms, and targets for drug development. In one study, hiPSC-derived neurons from four idiopathic SZ patients exhibited defects in connectivity and altered gene expression, which were partially reversed by the antipsychotic drug Loxapine [38]. In other studies, hiPSC-derived neurons from idiopathic SZ patients have shown enhanced oxidative stress [39], abnormal responses to environmental stressors [40] and deficits in synapse maturation [36,39,41••]. HiPSCs have also been generated from BD patients and neurons derived from these iPSCs exhibited increased levels of CXCR4 and changes in expression of genes critical for neuroplasticity, including WNT pathway components and ion channel subunits [42•]. Furthermore, transcripts involved in Ca^{2+} signaling and telencephalic neuronal fate are altered in BD patient iPSC-derived neurons, whereas lithium pretreatment of these neurons significantly ameliorated the phenotypes [43•]. These studies highlight that the same disease can have different genetic origins [36,38] and that different psychiatric disorders [13••,38,42•] can share common molecular signatures, such as dysregulation of gene expression related to synaptic transmission and WNT pathways [13••,38,42•,44].

One interesting approach is to stratify patients based on their responsiveness to specific treatments, for example, lithium responsiveness in BP and clozapine responsiveness in SZ. In a recent study, it was shown that a hyperexcitability phenotype was selectively reversed by lithium treatment only in hippocampal neurons derived from BP patients who also responded to lithium treatment [45••]. This represents a proof-of-principle for patient-specific iPSC model systems for drug testing and screening and the potential for personalized medicine.

Selection of cell types for phenotypic analysis

In addition to careful patient selection, it is critical to identify the appropriate cell type to study. Once identified, generating region-specific and disease-relevant cell types remains a significant challenge, but efficient protocols exist for direct differentiation toward glutamatergic, GABAergic, dopaminergic, and motor neurons, as well as astrocytes and oligodendrocytes (Figure 2).

Aberrant function of cortical glutamatergic neurons has been implicated in many psychiatric disorders. For SZ, the glutamate hypothesis was first proposed about 30 years ago based on the observation that psychotomimetic agents phencyclidine and ketamine induce 'schizophrenia-like' symptoms in healthy individuals by blocking neurotransmission at NMDA-type glutamate receptors [46]. Since then, postmortem neurochemistry, *in vivo* human brain imaging, and clinical pharmacology have further implicated glutamatergic dysfunction in SZ [47]. Critical glutamatergic genes such as GRM3 and GRIN2A, and Ca^{2+} channel subunits (CACNA1C and CACNA1), were found to be associated with SZ in recent GWAS [9]. Differentiation of hiPSCs into cortical glutamatergic neurons has been achieved via first manipulating bone morphogenetic protein (BMP), Wnt/β-catenin and TGF-β/activin/nodal pathways, followed by sequential specification into cortical layer identities, including early-born deep-layer $TBR1^+/CTIP2^+$ neurons and later-born upper-layer $BRN2^+/CUX1^+/SATB2^+$ neurons (Figure 2) [22,36,48••,49–51].

Changes in cortical GABAergic transmission are also associated with major psychiatric disorders [52–56] and several groups have successfully generated GABAergic interneurons with mature physiological properties [57•,58,59,60••]. HiPSCs are first patterned to $NKX2.1^+$ medial ganglionic eminence progenitors and then into interneurons expressing both pan-GABAergic (GAD1, SLC32A1, and SLC6A1) and specific subtype markers, including somatostatin, parvalbumin (PV), calretinin, calbindin and neuropeptide Y (Figure 2). Interestingly, GABAergic interneurons take longer to mature than glutamatergic neurons in culture, mimicking endogenous human neural development.

Aberrant dopaminergic (DA) transmission has also been linked to neuropsychiatric disorders [61,62]. Excess DA transmission in subcortical regions may lead to hyperstimulation of D2 receptors and positive symptoms, while hypoactive DA transmission at D1 receptors in the prefrontal cortex may contribute to cognitive impairments and negative symptoms. This DA hypothesis is supported by pharmacological, postmortem, and imaging data [63–65], and by recent GWAS data showing that the DRD2 receptor, a current pharmacological target, is associated with SZ [9]. Multiple studies have developed and optimized efficient protocols to differentiate hiPSCs into midbrain DA neurons [66–69]. In particular, Kriks *et al.* used a floor-plate-based strategy for the derivation of human midbrain DA neurons by patterning hiPSCs into floor-plate precursors with activation of SHH and Wnt/β-catenin signaling,

Figure 2

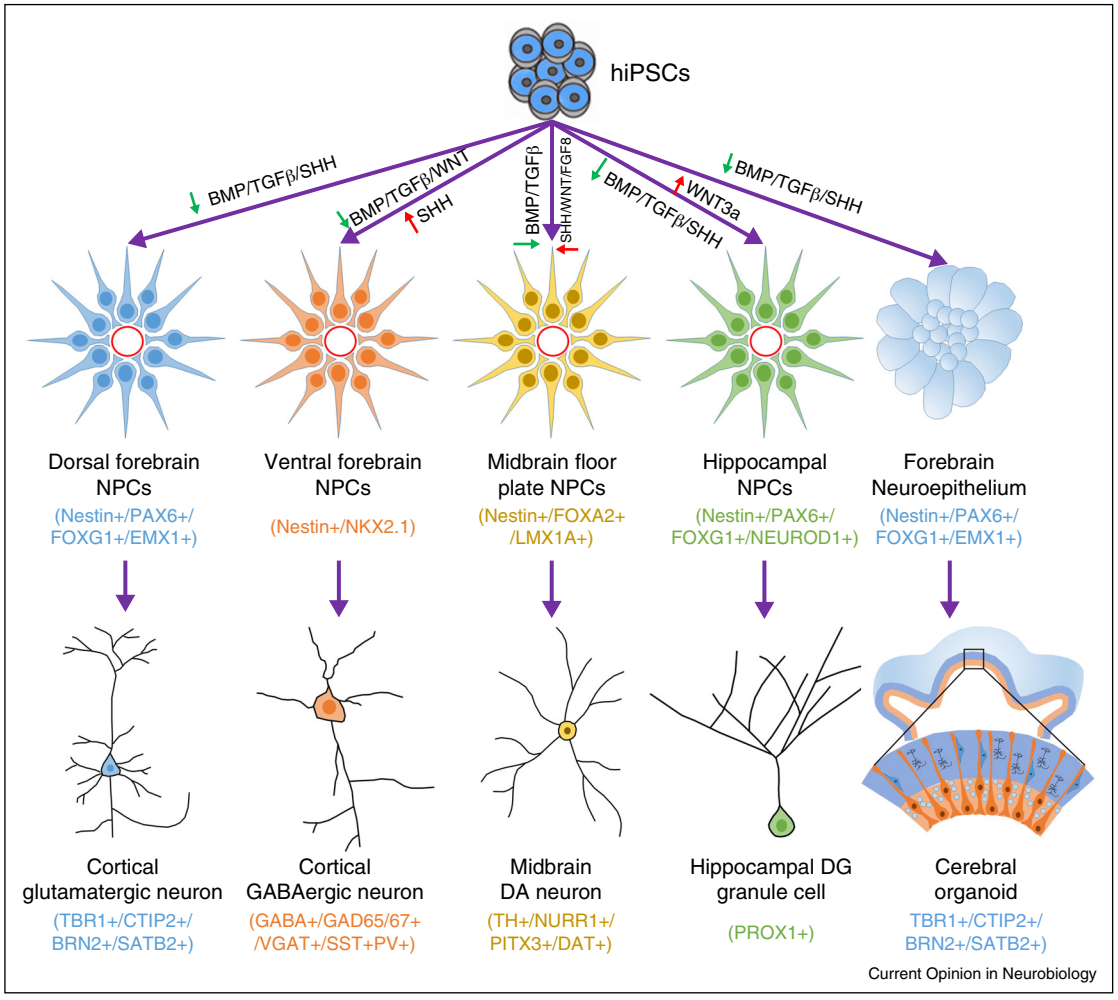

Diagram of differentiation of neuronal subtypes and 3D organoids *in vitro*. Differentiation of disease-related neuronal subtypes or 3D organoids from hiPSCs have been established. By manipulating morphogen pathways such as BMP, TGFβ, WNT, SHH pathways as well as other growth factors such as FGFs, human iPSCs could be induced into brain region-specific neural progenitor cells (NPCs), including dorsal forebrain NPCs, ventral forebrain NPCs, midbrain floor plate NPCs, and hippocampal NPCs. These area-specific NPCs could be further differentiated into different neuronal subtypes for disease modeling and drug screening, including cortical glutamatergic neurons or cerebral organoids ($TBR1^+/CTIP2^+/BRN2^+/SATB2^+$), cortical GABAergic neurons ($GABA^+/GAD65/67^+/VGAT^+/SST^+/PV^+$), midbrain DA neurons ($TH^+/NURR1^+/PITX3^+/DAT^+$), and hippocampal granule cells ($PROX1^+$).

which were further differentiated into functional midbrain neurons expressing the DA neuronal markers tyrosine hydroxylase (TH) and pituitary homeobox 3 (PITX3) [67]. Importantly, the midbrain DA neuron identity was further confirmed by extensive gene expression analysis, electrophysiological characterization, biochemical assessment, and *in vivo* transplantation.

Other disease-relevant cellular subtypes include hippocampal neurons [41••]. Dentate gyrus granule neurons derived from SZ hiPSCs exhibit lower levels of NEUROD1, PROX1, and TBR1, and reduced neuronal activity [41••]. Generation of astrocytes [70–73] and oligodendrocytes [74,75] from patient-derived iPSCs have not been studied extensively but could have a direct impact on neural circuitry and/or exert a non-cell-autonomous effect of genetic risk factors on different neuronal subtypes.

Currently, there are no established protocols to generate entirely pure populations of a specific cell type, much less for cortical layer-specific neurons or GABAergic subtypes. Cortical layer-specificity is relevant because the superficial layers (II–IV) may be particularly affected by psychiatric conditions. Similarly, among GABAergic subtypes, PV^+ interneurons have been frequently implicated in psychiatric pathology but there are no published protocols that can efficiently enrich for this subtype [58,59,60••,76,77]. To eliminate heterogeneity during cellar phenotyping, specific cell types can be fluorescently labeled via reporter gene

expression [41••,59,60••]. Genome editing via CRISPR/Cas9 can also be used to add a reporter (i.e. GFP) into endogenous cell subtype-specific gene loci. One new approach to analyze specific cell types is by transcriptomic analysis at the single cell level, which allows for certainty in cell identification and avoids techniques that might compromise transcriptome information, such as viral-mediated labeling, introduction of promoter-specific reporter lines, or cell sorting [78].

In most hiPSC studies, 2D cell cultures have been used, which do not reflect the complex 3D environment of endogenous brain formation. Transplantation of hiPSC-derived NPCs or neurons to the mouse brain provides an *in vivo* setting for human neurons to develop and functionally integrate into neuronal circuitry [41••,48••,58,67,79]. Recently, hiPSCs have been induced to form 3D cerebral organoid structures that recapitulate features of developing organs and are amenable to experimentation and drug testing [80–82]. Unlike rodents, developing embryonic human brains contain specialized outer radial glia cells that account for much of the evolutionary increase in cortical size and complexity [83]. Notably, such features are present in cerebral organoids derived from human iPSCs, but not from mouse iPSCs [84,85••,86••,87••]. Recently, hiPSC-derived cerebral organoids have been used to investigate mechanisms of severe idiopathic ASD and revealed accelerated cell cycles and overproduction of GABAergic inhibitory neurons [85••].

Drug development with hiPSCs

Patient iPSC-derived neurons have been demonstrated to exhibit disease-relevant phenotypes and respond to existing drugs *in vitro*, such as gentamicin-mediated effects on RTT-patient neurons [15], loxapine-mediated effects on SZ-patient neurons [38], roscovitine-mediated effects on TS-patient neurons [22], and IGF1-mediated effects on 22q13-deletion syndrome neurons [29••]. Importantly, a proof-of-principle study for familial dysautonomia demonstrated the feasibility of screening for novel therapeutic compounds with hiPSC-based cellular phenotypes [88]. Recently, large-scale high-throughput screening (HTS) assays have been established for NPCs based on Wnt/β-catenin signaling [89]. In addition to phenotypic assays, a novel pathway-centric HTS screen using the latest deep-sequencing technology may offer advantages over conventional chemical screening strategies [90]. Instead of focusing on one gene, this approach screens for patterns of endogenous gene expression changes with multiple targets simultaneously, enabling large-scale and quantitative analysis of gene matrices associated with specific disease phenotypes [90].

HiPSC drug discovery platforms still face several challenges. First, cell-type heterogeneity reduces the chances of identifying a positive hit, illustrating the need for better differentiation protocols. Second, overall culture conditions need to be controlled and standardized. Third, phenotypic assays need to be robust and should be disease-relevant. Finally, animal models are likely still required for validation of the positive hits from hiPSC-based screening platforms. But even with these challenges, this technology holds great promise as a new translational platform for drug testing using human neurons.

Summary

Patients exhibit significant inter-individual variability in their responses to psychoactive drugs, and family members who carry the same genetic mutation can develop different diseases. With recently developed techniques, including deep sequencing, efficient hiPSC generation, neuronal subtype differentiation, genome-editing, and HTS assays and screens, patient-specific hiPSCs have the potential to make personalized medicine feasible for heterogeneous and genetically complex psychiatric disorders. While there are still many challenges in directly translating cell-based findings into the clinic, the ability to investigate specific neural subtypes at single-cell resolution, to study disease mechanisms in a 3D cellular structure recapitulating organized features of human brain, and to establish a scalable HTS platform for drug discovery, all support the idea that hiPSC research could lead to a better understanding of disease mechanisms and more targeted treatments in the near future.

Conflict of interest statement

G-lM and HS were scientific co-founders of JuvoBio Pharmaceuticals Inc.

Acknowledgements

We apologize for not being able to cite all many original studies because length limit. The research in the authors' laboratories was supported by NIH (NS095348 to G-lM, and MH087874), NARSAD to KMC, and Maryland Stem Cell Research Fund to ZW, KMC, HS and G-IM.

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- •• of outstanding interest

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